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PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

Topical Parasiticide Formulations and Methods of Treatment

Name and Address of Applicant:

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This invention is best described in the following statement:

Topical Parasiticide Formulations And Methods Of Treatment

Technical Field

This invention relates to formulations for administration of benzimidazoles or salicylanilides with macrocyclic lactones to livestock for the control of endo- as well as ecto-parasites, methods for dosing livestock with such formulations, and methods for controlling and/or preventing diseases or parasite infection in livestock.

Background Art

A number of formulations including active components, such as therapeutic, prophylactic and/or bioactive substances, for the treatment and/or prophylaxis of diseases or parasite infection in livestock are known, including tablets and solutions for oral administration, injectable solutions, treated collars and ear-tags, and topical means including pour-on and spot-on formulations.

Many of the early formulations were intended for topical treatment/prophylaxis of ectoparasite-related conditions, being designed to spread the active component over the skin and/or hair surfaces of the animal, not to administer the active component(s) to the bloodstream of the animal being treated. More recently, endoparasiticide pour-on formulations for administration of particular active agents, including macrocyclic lactones, to the bloodstream of domestic animals such as sheep and cattle, have been developed, and these have the advantage over other administration forms, such as oral drenches and injection, of being easily applied to animals in a relatively accurate amount.

Known pour-on and spot-on formulations for endoparasiticide treatment generally utilise a non-aqueous delivery system for administering active components to animals, many of the active ingredients of interest being substantially water insoluble (particularly macrocyclic lactones, levamisole base, benzimidazoles), and it was believed that dissolution of the parasiticide was necessary in order for the parasiticide to become systemically absorbed).

Commercial ectoparasiticide products are available as both solvent based and aqueous based formulations. Water insoluble actives have been formulated as aqueous suspension pour-on formulations e.g. deltamethrin (Synthetic pyrethroid) for treatment of lice on sheep (Clout S®) and cattle (Arrest®) and

diflubenzuron (insect growth regulator, or IGR) for lice on sheep (Magnum IGR®). These treatments are characterised by the low levels of actives found in tissues following treatment, therefore, there is little penetration of active through the skin layer. Solvent based formulations containing the water insoluble IGR, triflumuron (e.g Zapp®) for lice control on sheep are also available. At an equivalent dose rate to the aqueous based formulations, these solvent based formulations lead to higher tissue residues immediately after treatment. This supports the assertion that a water insoluble active will be more easily systemically absorbed if it is solubilised in the formulation.

By 'water-insoluble' it is meant that the water-solubility is insufficient for an effective amount of an endoparasiticide to be dissolved in a commercially viable dose of a water-based pour-on formulation. Practically, a dose of pour-on formulation should not be much more than 1.0mL /10kg bodyweight (for ease of application and to prevent run off). At this rate a 500kg beast would receive a 50mL dose, therefore, a 2.0mL/10kg dose is not practical as many animals weigh much more than 500 kg.

Benzimidazoles and macrocyclic lactones are important classes of agents for the treatment or prevention of a number of important endoparasites of livestock, including liver fluke disease caused by the liver parasite *Fasciola hepatica*, and nematodes such as *Cooperia*, *Ostertagia*, *and Trichostrongylus* species.

Liver fluke disease may be acute or chronic and is best recognised in sheep and cattle.

Triclabendazole is a particularly effective benzimidazole, being the most effective drug currently available against all stages of *Fasciola hepatica*, destroying the early immature and immature fluke migrating through the liver as well as the adults in the bile duct.

Salicylanilide compounds form another important class of agents for control of endoparasites, particularly *Fasciola hepatica*, and nematodes such as *Haemonchus* species. The salicylanilide oxyclozanide is effective against adult liver fluke (*Fasciola hepatica*) and immature paramphistones migrating in the intestine of cattle and the young flukes in the rumen and reticulum. Oxyclozanide

is highly insoluble in water and is administered to animals in an aqueous suspension formulation by oral dosing.

Commercial endectocide pour-on products containing the avermectins ivermectin (Paramax®, Ivomec® Cattle Pour-On), moxidectin (Cydectin®) and doramectin (Dectomax®) are currently available for treatment of cattle for the control or prophylaxis of a number of endo- as well as ectoparasites such as lice, flies and ticks. These formulations, however, require significantly higher administration rates of the active component, as compared to oral drenching techniques, typically at least two times the oral drenching rates in order to achieve effective blood concentrations of the active ingredient in the animal, and to achieve the same efficacy of treatment. For example, ivermectin oral solution for cattle (registered in New Zealand) has a dose rate of 200 micrograms ivermectin/kg bodyweight whereas Ivomec Cattle Pour On has a dose rate of 500 micrograms ivermectin /kg bodyweight.

Pour-on or spot-on formulations for successful and/or efficient administration of triclabendazole to the bloodstream of animals are not commercially available, treatment of liver fluke in cattle with anthelmintics such as triclabendazole being generally carried out by oral drenching with a commercial product, for example Fasinex® 120 (120 g/L triclabendazole, Novartis), as well as by injection (Ivomec Plus® Antiparasitic Injection for cattle which also controls adult liver fluke).

By 'efficient delivery' it is meant that the active agent is administered at a rate approximating oral dosage rates up to about twice normal oral dosage rates to give effective blood concentrations and equivalent efficacy.

Disclosures relating to benzimidazole/anthelmintic pour-on formulations have been made.

US 5,925,374 discloses an anthelmintic pour-on formulation in which benzimidazoles are preferably present in a microsuspension formulation. The commercially available Bomatak White Stripe®, which conforms with the formulations described in this document, contains 7.5 % oxfendazole and is claimed to give prolonged release of the active which leads to enhanced anthelmintic efficacy. However, the active agent needs to be applied to an animal at over twice the oral dose rate to give effective blood concentrations of

the active ingredient and equivalent efficacy. The nematode efficacy (Faecal Egg Count) data provided in this document are based on oxfendazole. The described maximum oxfendazole blood serum levels in cattle are reported as 0.2µg/mL at a dose rate of 10mg oxfendazole /kg bodyweight (i.e 2.2 times the standard oral dose rate of 4.5mg/kg).

US 4,479,960 discloses pour-on formulations comprising anthelmintics, including thiazoles or benzimidazoles, in a solvent carrier. Some of the solvents for use in these formulations are flammable and/or carcinogenic (for example, xylene, benzene, toluene), and may also cause skin irritancy. Efficacy against fluke (using a substituted benzimidazole) is described. However, dose volumes described are high and not commercially practical, also requiring administration of efficacious levels of benzimidazole which would constitute five to ten times the effective drench dose rates for triclabendazole.

IE 920150 discloses anthelmintic pour-on formulations containing at least one active ingredient selected from a group including benzimidazoles. The active agent is not fully solubilised, but provided in fine particle dispersion, in a penetration-promoting agent. The penetrating-promoting agents described are triglycerides of saturated vegetable fatty acids, esters of long chain acids and long chain alcohols. Surfactants are included as emulsifiers. Efficacy of the formulations is only described against nematodes.

Pour-on or spot-on formulations of salicylanilide derivatives are not currently available, usually being administered to livestock by oral drench.

It would be highly desirable, in order to provide broad spectrum protection against endoparasites and ectoparasites, to be able to efficiently administer water insoluble compounds such as benzimidazoles or salicylanilides in combination with macrocyclic lactones to the bloodstream of animals by a single convenient topical application rather than by oral administration.

International Publication No. WO00/61068 (PCT/NZ00/00053) discloses triclabendazole, optionally in combination with a macrocyclic lactone, dissolved in at least one solvent, preferably administered as a pour-on formulation for control of liver fluke. Efficacy data supplied (based on a low natural infection fluke challenge, mean of 20), however, shows that the formulation was applied at 2.5 times the dose of a standard oral drench rate to give equivalent efficacy. Also,

two of the solvents described, xylene and toluene, are highly flammable. The reported triclabendazole content of the formulation after 345 days storage at ambient temperature is 7.5 % lower than the initial assay, although there is no decrease in the abamectin content. Solvent based formulations of ivermectin can break down rapidly unless suitably stabilised.

A solvent-based topically administered formulation of the salicylanilide closantel with the macrocyclic lactone ivermectin, for the control of parasites, has been described in US patent No. 6,340,672. The maximum concentration of active agents described in the examples of this document is 0.5%w/v for ivermectin and 5%w/v for closantel. At these concentrations, unacceptably large volumes of the formulations (from a practical viewpoint) would need to be poured onto the animals in order to achieve effective blood concentrations of the active agents.

WO 00/74489 (PCT/NZ00/00087) discloses biocidal compositions, including pour-on formulations which are water-in-oil (soyabean) emulsions stabilised with an emulsifying agent. The formulations comprise the water-soluble anthelmintic levamisole (as the hydrochloride salt) and a macrocyclic lactone (abamectin or ivermectin), optionally in combination with a benzimidazole (oxfendazole). Only low levels of benzimidazole are present in the formulations disclosed in this document (up to 5% w/v oxfendazole in an oral drench formulation), and only one pour-on formulation comprising a benzimidazole (2.26%w/v oxfendazole) and a macrocyclic lactone (0.1%w/v abamectin) is disclosed. Whilst this pour-on formulation is described as delivering the levamisole to the bloodstream of cattle with efficiency similar to oral drench administration, the macrocyclic lactones and benzimidazoles were delivered with low efficiency and a commercially unpractical volume of this formulation would be required to be applied to animals in order to achieve effective blood concentrations of these actives.

Objects of the Invention

It is an object of this invention to provide a topical formulation capable of efficient delivery of a benzimidazole or salicylanilide, in combination with a macrocyclic lactone to the bloodstream of an animal for broad spectrum control of endoparasites such as liver fluke and nematodes in animals such as sheep and cattle with a single, easily applied topical formulation.

Summary of the Invention

It has now been surprisingly found that a benzimidazole or a salicylanilide, in combination with a macrocyclic lactone, can be formulated into a stable aqueous micellar composition which, when applied topically to an animal, efficiently delivers the active constituents to the bloodstream of the animal and provides effective protection against infestation by endoparasites such as liver fluke and nematodes.

Thus, the present invention provides an aqueous micellar formulation comprising a first active agent selected from benzimidazoles, salicylanilides and active derivatives or salts thereof in combination with a second active agent selected from macrocyclic lactones or active derivatives or salts thereof, said formulation being for topical application to animals for the control of internal parasites and also comprising, per litre of formulation:

from about 100 to about 400g veterinary acceptable surfactant(s);
from about 200 to about 750g veterinary acceptable water-miscible solvent(s); and
from about 50 to about 350g water.

Surprisingly, it has also been found that the stability of aqueous micellar formulations of the invention can be improved by inclusion of a stabiliser selected from anionic surfactants such as sodium dodecyl sulphate (SDS) and/or buffering agents such as soluble phosphates and/or dibasic phosphates.

Thus, in a preferred aspect of the invention, the aqueous micellar formulation comprises a stabiliser selected from anionic surfactants or buffering agents, or mixtures thereof. Preferably the stabiliser is a linear alkyl sulphate, such as sodium dodecyl sulphate, or one or more phosphates/ dibasic phosphates, or mixtures thereof.

In a preferred embodiment, there is provided an aqueous micellar formulation comprising a benzimidazole in combination with a macrocyclic lactone, said formulation being for topical application to animals for the control of internal parasites and also comprising, per litre of formulation:

about 100 to about 300g polyoxyalkylene sorbitan fatty acid ester surfactant;

about 300 to about 650g alkylene glycol ether selected from alkylene or dialkylene glycol monoalkyl ethers or combinations thereof;
about 10 to about 100g polyethylene glycol;
about 5 to about 50g stabiliser; and
about 50 to about 350g water.

In a particularly preferred aspect of this embodiment, the formulation comprises, per litre formulation:

about 180 to about 240g triclabendazole;
about 5 to about 10g macrocyclic lactone or an active derivative or salt thereof;
about 150 to about 250g polyoxyethylene (20) sorbitan monolaurate;
about 450 to about 550g diethylene glycol monobutyl ether;
about 20 to about 50g PEG 200;
about 20g sodium dodecyl sulphate; and
about 100 to about 200g water.

The invention also provides a method of treating or preventing a diseased or parasite infested state in a mammal, comprising topically administering to said mammal a micellar formulation according to the invention.

Typically the diseased or infested state is related to liver fluke, such as caused by *Fasciola hepatica*, and nematodes such as *Cooperia*, *Ostertagia*, *Trichostrongylus* and *Haemonchus* species or combinations thereof.

Even more typically the diseased or infested state to be treated or prevented is a disease or infested state of cattle or sheep, more typically cattle.

Surprisingly, it was found that the location and size of the region of topical administration of the formulations was important for efficiency of permeation of the active agents across the skin into the bloodstream.

Thus, in a preferred aspect of the methods of treatment, the formulation is applied in a band along the lower portion of the back of the mammal.

Preferably, so as to maximise efficiency of delivery of the active agents to the bloodstream of the animal, the formulation is applied to the animal over as small

a region as possible while avoiding run-off of the formulation so as to maximise the concentration of active agents per cm^2 of animal surface.

In another preferred aspect of the methods of treatment, the formulation is sprayed onto the back of the animal.

Where the animals to be treated are cattle, the formulation is preferably applied to the flat part of the back, typically the last third of the animal, and most typically starting from the thoracic vertebrae and proceeding towards the rump of the animal. Typically, about 24mg benzimidazole/salicylanilide and about 0.75mg macrocyclic lactone are applied per kilogram animal. Typically the band of formulation applied will be from about 5 to about 15cm wide and, depending on the size of animal, about 20-40cm long, and even more typically the formulation is sprayed onto the back of the animal and the height of the source of spray relative to the back of the animal is maintained at about 5-10cm.

As used herein the term "treating or preventing", refers to any and all uses which remedy or prevent a diseased or infested state or symptoms, or otherwise prevent, hinder, retard, or reverse the progression of disease/infestation or other undesirable symptoms in any way whatsoever. "Infestation" and corresponding derived terms relate to infestation by endo- and/or ecto-parasites.

An "effective amount", as referred to herein, includes a non-toxic therapeutic or prophylactic amount of an active agent to provide the desired effect. The "effective amount" will vary from subject to subject depending on one or more of a number of factors amongst, for example, the particular agent being administered, the type and/or severity of a condition being treated, the species being treated, the weight, age and general condition of the subject and the mode of administration. For any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation. Also, extensive literature is available for many known active agents through, for example, manufacturers' catalogues, the Internet, scientific journals and patent literature, including effective amounts for administration to target animals.

Typically, "effective amount" refers to an amount of active agent sufficient to result in one or more or the following: recession/reduction in the extent of a disease/infestation; inhibition of disease/infestation growth or progression; cessation of disease/infestation growth or progression; prevention of

disease/infestation; relief of disease/infestation-imposed discomfort; or prolongation of life of the animal having the disease.

As used herein, the term "comprising" means "including principally, but not necessarily solely". Variations of the word "comprising", such as "comprise" and "comprises", have correspondingly varied meanings.

Detailed Description of the Invention

Aqueous Micellar formulations

The present invention is based on the finding that hydrophobic active agents such as benzimidazoles and salicylanilides can be provided in a formulation for topical administration along with therapeutic amounts of a macrocyclic lactone for efficient delivery of both the benzimidazole/salicylanilide and the macrocyclic lactone to the bloodstream of the animal for effective control of endoparasites such as liver fluke and nematodes. It has also been found by the present investigations that efficiency of delivery of the active agents to the bloodstream of a mammal is affected by the topical location of application of the formulation, minimising the area of the skin to which the active agents are applied and/or use of formulations having elevated concentrations of the active agents. The formulations of the present invention surprisingly allow for elevated concentrations of benzimidazole(s) or salicylanilide(s), and macrocyclic lactones to be provided in a single composition for efficient delivery of the active agents to the bloodstream of a mammal by topical administration.

The formulations are aqueous micellar compositions, comprising elevated levels of the active agents and, per litre of formulation:

from about 100 to 400g veterinary acceptable surfactant(s);
from 200 to 750g veterinary acceptable water-miscible solvent(s); and
from 50 to 350g water.

Advantageously, the surfactant is non-ionic and selected from sorbitan esters, polyoxyalkylated sorbitan esters, polyoxyalkylated alkyl ethers, polyoxyalkylated fatty alcohols, polyoxyalkylated fatty acids, polyalkylene glycol esters, polyoxyalkylated derivatives of castor oil, polyglycerol esters, copolymers of ethylene oxide and propylene oxide; amine ethoxylates; alkyl phenol ethoxylates; alkyl polysaccharides; or combinations thereof, although the

surfactant may also be, or include, anionic surfactants selected from linear alkylbenzene sulphonates; C12 to C16 alcohol sulphates; C12 alkoxypolyethanoxy sulphates; alkyl phosphates and phosphonates or combinations thereof.

Preferred surfactants are selected from polyoxyalkylated fatty alcohols and polyoxyethylene sorbitan- or sorbitol- fatty acid esters or combinations thereof, and particularly preferred are polyoxyethylene sorbitan- or sorbitol- fatty acid esters.

Generally the polyoxyalkylene sorbitan- or sorbitol- fatty acid esters are polyoxyethylene sorbitan fatty acid esters. Polyoxyethylene sorbitan fatty acid esters such as those of the Ecoteric® series are preferred. Especially preferred polyoxyethylene sorbitan fatty acid ester surfactants are polyoxyethylene (20) sorbitan monolaurate (Ecoteric® T 20) and polyoxyethylene (20) sorbitan monooleate (Ecoteric® T 80).

Typically the polyoxylated fatty alcohols are polyalkylene oxide derivatives of natural or synthetic alcohols, and those of synthetic alcohols such as provided by the Teric® series are preferred. Especially preferred is Teric® BL8.

Generally, the amount of surfactant used in the formulation ranges from about 100 to about 400g/L, typically about 100 to about 300g/L, more typically about 150 to about 300g/L, even more typically about 150 to about 250g surfactant, and even more typically about 175 to about 225g/L, preferably about 200g/L, based on the total amount of formulation.

The water-miscible solvent(s) may be selected from: ethanol; isopropanol; benzyl alcohol; glycol ethers; liquid polyoxyethylene glycols; or a mixture of at least two of these solvents.

Particularly preferred water-miscible solvents are the glycol ethers, and particularly in combination with a liquid polyethylene glycol. A particularly preferred polyethylene glycol is PEG 200.

Generally, the glycol ethers are alkylene glycol alkyl ethers, including ethylene glycol monoethyl ether, ethylene glycol monomethyl ether, propylene glycol monomethyl ether (Glysolv PM®), dipropylene glycol monomethyl ether,

diethylene glycol monoethyl ether (Ethyl di Glysolv®), diethylene glycol monobutyl ether (Butyl di Glysolv® or Butyl Digol®), and diethylene glycol diethyl ether and the like. Particularly preferred glycol ethers are diethylene glycol monoethyl ether (Ethyl di Glysolv®) and/or diethylene glycol monobutyl ether (Butyl di Glysolv® or Butyl Digol®).

Generally the amount of water-miscible solvent(s) used in the formulation ranges from about 200 to about 750g/L, typically about 300 to about 650g/L, more typically about 300 to about 550g/L and even more typically about 400 to about 550g/L, preferably about 450 to about 550g/L, based on the total amount of formulation, but will vary depending on the particular solvent(s) used and the amount of active agents to be included in the micellar formulation.

Where, according to a preferred aspect of the invention, the formulation comprises both a glycol ether and a liquid polyethylene glycol, the amount of glycol ether used in the formulation typically ranges from about 350 to about 650g/L, more typically about 400 to about 600g/L and even more typically about 450 to about 550g/L, preferably about 450 to about 500g/L, based on the total amount of formulation. The amount of liquid polyethylene glycol used in the formulation typically ranges from about 10 to about 100g/L, more typically from about 20 to about 70g/L, even more typically from about 20 to about 50g/L, preferably about 30g/L, based on the total amount of formulation.

Generally the amount of water used in the formulation ranges from about 50 to about 350g/L, typically about 100 to about 300g/L, more typically about 100 to about 250g/L and even more typically about 150 to about 200g/L, preferably about 150 g/L, based on the total amount of formulation.

Examples of suitable benzimidazoles include: 2-(4-thiazolyl)-1H-benzimidazole, known as thiabendazole; [5-(propylthio)-1H-benzimidazol-2-yl]carbamic acid methyl ester, known as albendazole; [5-(propylsulfinyl)-1H-benzimidazol-2-yl]carbamic acid methyl ester known as albendazole sulfoxide or albendazole oxide; [2-(4-thiazolyl)-1H-benzimidazol-5-yl]carbamic acid 1-methylethyl ester, known as cambendazole; [5-(phenylthio)-1H-benzimidazol-2-yl]carbamic acid methyl ester, known as fenbendazole; (5-benzoyl-1H-benzimidazol-2-yl)carbamic acid methyl ester, known as mebendazole; [5-(phenylsulfinyl)-1H-benzimidazol-2-yl]carbamic acid methyl ester, known as is oxfendazole; (5-propoxy-1H-

benzimidazol-2-yl)carbamic acid methyl ester, known as oxibendazole; [5-(N-butyl)-1H-benzimidazol-2-yl]carbamic acid methyl ester known as parbendazole; methyl 5-cyclopropylcarbonyl-1H-benzimidazol-2-ylcarbamate known as cyclobendazole; methyl 5-(4-fluorobenzoyl)-1H-benzimidazol-2-ylcarbamate known as flubendazole; 5-chloro-6-(2,3-dichlorophenoxy)-2-(methylthio)-benzimidazole known as triclabendazole; and [5-(4-fluoro-phenylsulfonyloxy)-1H-benzimidazol-2-yl]carbamic acid methyl ester known as luxabendazole.

The benzimidazole antiparasitic agents are active against one or more of *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Bunostomum*, *Strongyloides*, *Trichuris*, *Oesophagostomum*, *Chabertia*, *Dictyocaulus*, *Moniezia* and *Fasciola* in sheep and against *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Bunostomum*, *Capillaria*, *Strongyloides*, *Trichuris*, *Oesophagostomum*, *Chabertia*, *Dictyocaulus*, *Moniezia* and *Fasciola* in cattle.

Particularly preferred as benzimidazole is triclabendazole.

Examples of suitable salicylanilide compounds for use in the control of *Fasciola* and *Haemonchus* species in livestock include oxyclozanide (3,3',5,5',6-pentachloro-2'-hydroxysalicylanilide), closantel (5'-chloro-4'-(4-chloro-alpha-cyanobenzyl)-3,5-diiodosalicyl-*o*-toluidide), rafoxanide (3'-chloro-4'-(4-chlorophenoxy)-3,5-diiodosalicylanilide), and niclosamide (2',5-dichloro-4'-nitrosalicylanilide), as well as clioxanide, brotianide and bromoxanide.

Salicylanilide derivatives, and their use for control of endoparasites in livestock, has been described in, for example, US patent numbers 3,914,418, 3,927,071, 3,989,826, 4,005,218, 4,025,647, "Veterinary Anthelmintics", J.H. Arundel, University of Sydney, Post Graduate Foundation in Veterinary Science, and the Merck Veterinary Manual (http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/191415.htm).

Oxyclozanide is a particularly preferred salicylanilide for use in formulations according to the invention.

Typically, the macrocyclic lactone(s) is/are selected from the group consisting of ivermectin (22,23-dihydroavermectin B_1 described in EP 295117), abamectin, avermectin A_{1a}, avermectin A_{1b}, avermectin A_{2a}, avermectin A_{2b}, avermectin B_{1a},

avermectin B_{1b}, avermectin B_{2a}, and avermectin B_{2b}. Also typically, the macrocyclic lactone may be selected from active derivatives of the naturally occurring avermectins, such as derivatives which have a group at the 25-substituent other than the isopropyl or (S)-sec-butyl groups, as set out in European patent applications 0214731, 0284176, 0308145, 0317148, 0335541 and 0340832. Also, typically, the macrocyclic lactone of the first aspect of the invention can include moxidectin (and derivatives disclosed in EP 259779A), doramectin and its analogues (described in EP0214731B), selamectin, eprinomectin, milbemycin including milbemycin oxime, milbemycin D (Antibiotic B41D) and its analogues (described in US3,950,360) and nemadectins (described in EP 170006A).

The macrocyclic lactone antiparasitic agents are active against one or more of *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Strongyloides*, *Trichuris*, *Oesophagostomum*, *Chabertia* and *Dictyocaulus* in sheep and against *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Oesophagostomum* and *Dictyocaulus* in cattle.

Particularly preferred as a macrocyclic lactone is ivermectin.

Generally, where present, the amount of benzimidazole used in the formulation ranges from about 90 to about 360g/L, typically about 90 to about 300g/L, more typically about 150 to about 300g/L, even more typically about 180 to about 270g/L and even more typically about 180 to about 240g/L, preferably about 240g/L, based on the total amount of formulation. Generally about 9 to about 36mg, typically about 9 to about 30mg, more typically about 15 to about 30mg, even more typically about 18 to about 27mg and even more typically 18 to about 24mg, preferably about 24mg of benzimidazole per kg bodyweight are applied topically to a mammal in a single dosage.

Generally, where present, the amount of salicylanilide used in the formulation ranges from about 125 to about 500g/L, typically about 160 to about 375g/L, more typically about 200 to about 350g/L, even more typically about 250 to about 350g/L, and even more typically about 300 to about 330g/L, preferably about 330g/L based on the total amount of formulation. Generally, about 12.5 to about 50mg of oxyclozanide, typically about 16 to about 37.5mg, more typically about 20 to about 35mg, even more typically about 25 to about 35mg, and even more

typically about 30 to about 35mg, preferably about 33mg of salicylanilide per kg bodyweight is applied topically to a mammal in a single dosage.

Generally the amount of macrocyclic lactone used in the formulation ranges from about 2.5 to about 15g/L, typically about 4 to about 12g/L, more typically about 5 to about 10g/L and even more typically about 6 to about 9g/L, preferably about 7.5g/L, based on the total amount of formulation. Generally about 0.25 to about 1.5mg, typically about 0.4 to about 1.2mg, more typically about 0.5 to about 1.0mg, even more typically about 0.6 to about 0.9mg, preferably about 0.75mg of macrocyclic lactone per kg bodyweight are applied topically to a mammal in a single dosage.

Advantageously, the aqueous micellar formulations according to the invention also comprise a stabiliser. Preferably the stabiliser is selected from anionic surfactants such as linear alkyl sulphates (for example, sodium dodecyl sulphate), linear alkyl benzene sulphonates (such as calcium dodecyl benzene sulphonate) and buffering agents, typically selected from soluble monobasic and/or dibasic phosphates.

Sodium dodecyl sulphate is typically used as a stabiliser in the formulation in the range of from about 10 to about 30g/L, more typically from about 10 to about 20g/L, based on the total amount of formulation; phosphates are typically used in the formulation in the range of from about 1 to about 10g/L, more typically from about 1 to about 5g/L, and more typically from about 1 to 2g/L, based on the total amount of formulation.

The aqueous micellar formulations may also include one or more further veterinary excipients, provided these do not destabilise the micellar formulation.

Veterinary acceptable excipients for use in preparing the formulations may include, for example: further solvents such as, for example, water immiscible solvents including glycol ether esters; viscosity modifiers/suspending agents, for example, gelatin, vegetable gums such as xanthan gum, cellulose derivatives (e.g. AVICEL microcrystalline cellulose, anionic or non-ionic cellulose ethers, such as carboxymethylcellulose), fumed silica (colloidal silicon dioxide), or polyvinylpyrrolidone polymers, and magnesium aluminium silicates such as VEEGUM, and mixtures of these.

Examples of suitable veterinary acceptable adjuvants include dyes.

Dyes enable the treated mammals to be distinguished from the untreated. The dyestuff may be dissolved, suspended or dispersed in the carrier. The nature of the colouring agent is unimportant and a wide variety of suitable dyes and pigments will be known to the skilled person. The colouring agent may be soluble or insoluble in water. Generally, however, the dyestuff will be biodegradable so as to fade and not permanently mark the skin or fleece. Some examples of suitable dye agents include: Brilliant Blue FCF (Hexacol Brilliant Blue), Fast Scarlet Pigment 3610.

Processes for the preparation of micellar formulations of the invention

The micellar formulations according to the invention may be prepared by methods and techniques known to those of skill in the art.

Typically the formulations may be made using a simple process:

Step 1. Charge 80% of the total volume of water-miscible (non flammable) solvent and the surfactant to a manufacturing vessel. Heat to 40 – 75°C (flammable solvents such as ethanol and isopropanol, whether added as major water-miscible solvent or as a minor component should be used at ambient temperature).

Step 2. Add the benzimidazole or salicylanilide incrementally with continued stirring and heating until dissolved.

Step 3. Add sequentially the water, and optionally stabilisers and dye, stirring until dissolved.

Step 4. Cool to room temperature with continued stirring.

Step 5. Add the macrocyclic lactone incrementally with stirring until dissolved (also, if flammable solvents such as ethanol or isopropanol are to be added as co-solvents, they should be added here).

Step 6. Add the remaining solvent to volume.

Methods of Treatment and/or prevention of diseases or infestations

The formulations according to the invention may be used for the treatment and/or prevention of diseases or infestations by endoparasites in mammals, typically in

livestock such as sheep or cattle, by applying the formulation(s) to the back of the mammal. Important diseases/infestations which may be controlled include liver fluke, nematodes and lice in sheep and cattle and buffalo fly and ticks on cattle.

It was found that optimal uptake of the active agents into the bloodstream of treated mammals occurred when the formulations were applied to a region starting from the flat part of an animals back – approximately at the location of the thoracic vertebrae – and working towards the rump of the animal, effectively resulting in application of the formulation to the last third of the mammal's back. This mode of application was found to be significantly more effective than application starting at the neck.

Efficiency of delivery of the active agents to the bloodstream of a mammal was also found to be greatest where the surface area to which the formulation is applied was minimised, while avoiding run-off of the formulation, so as to maximise the concentration of active agents per cm^2 of animal surface, typically covering an area of about 100 to about 400cm^2 for cattle and about 100cm^2 for sheep.

Typically the formulation is applied by spray onto the mammal's back, preferably from a constant height relative to the mammal's back.

For cattle, the band of formulation is typically applied starting from the thoracic vertebrae and proceeding towards the rump of the animal. Typically, about 24mg benzimidazole and about 0.75mg macrocyclic lactone are applied per kilogram mammal. Preferably this amount of active agents is applied to the mammal in about 0.05 to about 0.1mL per kg mammal, and in a band from about 5 to about 15cm wide. In weaned calves typically weighing from about 100 to about 180kg per head, good results were obtained by spraying about 10 to about 18mL formulation onto the backs of the animals, starting from the thoracic vertebrae and working towards the animals' rumps, from a constant height of about 15cm relative the backs of the animals, resulting in an applied band of formulation about 10 to about 15cm wide and about 20cm long.

Preferred forms of the present invention will now be described, by way of example only, with reference to the following examples, including comparative

data, and which are not to be taken to be limiting to the scope or spirit of the invention in any way.

Examples

Example 1 – Aqueous micellar formulations, and processes for preparing them

1.1 Formulation A

Component	g/L
Triclabendazole	240
Ivermectin	7.5
Polyoxyethylene (20) sorbitan monolaurate (Ecoteric® T 20)	200
Polyethylene glycol 200 (PEG 200)	30
Water	150
Sodium dodecyl sulphate	20
Diethylene glycol monobutyl ether	475mL
Brilliant Blue FCF	0.16

1.2 Formulation B

Component	g/L
Triclabendazole	240
Ivermectin	7.5
Polyoxyethylene (20) sorbitan monolaurate (Ecoteric® T 20)	200
Polyethylene glycol 200 (PEG 200)	30
Water	250
Sodium dodecyl sulphate	20
Diethylene glycol monobutyl ether	475mL
Brilliant Blue FCF	0.16

1.3 Formulation C

Component	g/L
Triclabendazole	120
Ivermectin	5.0

Polyalkylene oxide derivative of synthetic alcohol (Teric® BL8)	200
Benzyl alcohol	30
Water	150
Diethylene glycol monobutyl ether	520mL
Dihydrogen sodium phosphate	7.84
Disodium hydrogen phosphate	0.91
Brilliant Blue FCF	0.16

1.4 Formulation D

Component	**g/L**
Triclabendazole	120
Ivermectin	5.0
Polyoxyethylene (20) sorbitan monooleate (Ecoteric® T 80)	200
Benzyl alcohol	30
Water	250
Propylene glycol monomethyl ether (Glysolv PM®)	420mL
Disodium hydrogen phosphate	0.91
Dihydrogen sodium phosphate	7.84
Brilliant Blue FCF	0.16

1.5 Formulation E

Component	**g/L**
Oxyclozanide	350
Ivermectin	7.5
Polyoxyethylene (20) sorbitan monooleate (Ecoteric® T 80)	200
Water	150
Sodium dodecyl sulphate	20
Diethylene glycol monobutyl ether	520mL
Brilliant Blue FCF	0.16

Other stable aqueous micellar formulations according to the invention are described in Examples 2 and 3.

The formulations were prepared by the following procedure:

Step 1. Charge 80% of the total volume of water-miscible solvent and the surfactant to a manufacturing vessel. Heat to 40 – 75°C with stirring.

Step 2. Add the benzimidazole or salicylanilide incrementally with continued stirring and heating until dissolved.

Step3. Add sequentially the water, and optionally stabilisers and dye, stirring until dissolved.

Step4. Cool to room temperature with continued stirring.

Step 5. Add the macrocyclic lactone incrementally with stirring until dissolved.

Step 6. Add the remaining solvent to volume.

Example 2 – Pharmacokinetic studies

Materials and methods

Formulations according to the invention were tested for their efficacy in delivering benzimidazoles and macrocyclic lactones to the bloodstream of mammals (cattle), and compared to the efficacy in delivering these agents to animals' bloodstreams by standard commercially available drench (Fasinex 120®), and an experimental solvent-based triclabendazole/ ivermectin pour-on formulation.

Cattle (typically Hereford or Hereford cross) with either natural or artificially infected burdens of fluke and nematodes were used in pen and field trials. Within a given trial animals were allotted into treatment groups, each having similar mean weights and fluke and nematode burdens. Experimental treatments were applied along the backline using a commercially available backliner gun fitted with a plastic shroud to ensure correct delivery of the formulation according to the protocol.

Blood samples (plasma) were taken by venipuncture of the jugular vein at the designated time intervals. Analysis for triclabendazole and ivermectin residues in the plasma was carried out and reported by commercial contract laboratories .

Ivermectin was extracted from the plasma using acetonitrile and concentrated by evaporation. The sample was cleaned up by solid phase extraction (SPE)

chromatography and the ivermectin determined as the N-methyl imidazole derivative using reverse phase HPLC with fluorescence detection.

The triclabendazole was extracted from the plasma using ethyl acetate. Following concentration and SPE clean up, the triclabendazole and its sulphone and sulphoxide metabolites were analysed by reverse phase HPLC using UV detection.

Results

Initial feasibility studies for development of an efficient flukicide product were based on the pharmacokinetic profile of triclabendazole only. Although noting that the bioavailability of the active agents is always delayed after application as a pour-on formulation compared to a drench treatment, blood plasma levels for the experimental formulations were targeted at the maximum triclabendazole plasma levels (C_{max}) produced by the currently available flukicide, Fasinex® 120 (triclabendazole C_{max} 16.5µg/mL after 2 days) , when applied at a rate of 12mg/kg bodyweight.

Having reference to Table 1, the following results were obtained.

In a first feasibility trial (Hereford male weaner cattle, average weight of approximately 200kg, 2 animals per group), a solvent-based formulation (N-methyl pyrrolidone/ Butyl diGlysolv®, Formulation 1), triclabendazole was applied at 50mg/kg to achieve similar plasma levels as per the currently available flukicide, Fasinex® 120 (15.7µg/mL after 7 days). Such a dose rate is not commercially viable.

In a second feasibility trial (Hereford male and female weaner cattle, average weight of approximately 160kg, 3 animals per group) the triclabendazole dose rate was reduced to a more commercially acceptable level (12mg/kg). A surfactant (Teric® BL8) was added to Formulation 1 to improve the formulation's hide wettability to produce Formulation 2 (non aqueous micelle), and N-methyl pyrrolidine solvent was removed. Triclabendazole C_{max} (total metabolite) plasma levels achieved were low (2.0µg/mL).

Addition of 15 % water to Formulation 1 produced Formulation 3 (Formulation C described in Example 1.3 above, an aqueous micelle), and this increased the triclabendazole C_{max} achieved to 4.8µg/mL.

TABLE 1

Formulation and Type	Formulation Details	g or mL per litre	Dose Rate mg/kg	Plasma C_{max}	T_{max} days
1	Triclabendazole	250g	50	15.7µg/mL	7
	Ivermectin	2.5g			
Solvent based	N- Methyl pyrrolidone	400mL			
	Butyl di Glysolv®	575mL			
Control Fasinex 120	*120 g/L TCBZ*		12	16.5µg/mL	2
2	Triclabendazole	120g	12	2.0µg/mL	7
	Ivermectin	5.0g			
Non-aqueous	Teric® BL8	200g			
micelle	Benzyl alcohol	30g			
	Butyl di Glysolv®	650mL			
3	Triclabendazole	120g	12	4.8µg/mL	7
	Ivermectin	5.0g			
Aqueous	Teric® BL8	200g			
micelle	Water	150g			
	Benzyl alcohol	30g			
	Butyl di Glysolv®	520mL			
4	Triclabendazole	120g	12	8.7µg/mL	7
	Ivermectin	5.0g	0.5	1.3ng/mL	5
Aqueous	Teric® BL8	200g			
micelle	Water	250g			
	Benzyl alcohol	30g			
	Glysolv PM®	420mL			
	Dihydrogen Sodium Phosphate	7.84g			
	Disodium Hydrogen phosphate	0.91g			
5	Triclabendazole	120g	12	8.7µg/mL	7
	Ivermectin	5.0g	0.5	2.6ng/mL	2
Aqueous	Teric® BL8	200g			
micelle	Water	150g			
	Benzyl alcohol	30g			
	Glysolv PM®	520mL			
	Dihydrogen sodium Phosphate	7.84g			
	Disodium hydrogen phosphate	0.91g			

TABLE 1 (continued)

Formulation ID and Type	Formulation Details	g or mL per litre	Dose Rate mg/kg	Plasma C_{max}	T_{max} days
6	Triclabendazole	120g	12	15.9µg/mL	7
	Ivermectin	5.0g	0.5	2.8ng/mL	5
Aqueous micelle	Ecoteric® T20	200g			
	Water	250g			
	Benzyl alcohol	30g			
	Glysolv PM®	420mL			
	Dihydrogen Sodium Phosphate	7.84g			
	Disodium Hydrogen phosphate	0.91 g			
7	Triclabendazole	120g	12	12.9µg/mL	7
	Ivermectin	5.0g	0.5	3.0ng/mL	7
Aqueous micelle	Ecoteric® T80	200g			
	Water	250g			
	Benzyl alcohol	30g			
	Glysolv PM®	420mL			
	Dihydrogen Sodium Phosphate	7.84g			
	Disodium Hydrogen phosphate	0.91g			

In a further feasibility trial (Hereford female cattle, average weight of approximately 235kg, 3 animals per group), the water content in the formulation was increased to 25% and Butyl di Glysolv® was replaced with Glysolv PM®. The resulting Formulation 4, provided an increased triclabendazole C_{max} of 8.7µg/mL - almost double that achieved with Formulation 3. The ivermectin C_{max} achieved was 1.3ng/mL at 5 days.

A similar formulation, Formulation 5, had a water content of 15 %. Although the C_{max} for triclabendazole was almost the same, 8.6µg/mL, the C_{max} for ivermectin was 2.6ng/mL at 2 days.

Replacing Teric® BL8 in Formulation 4 with Ecoteric® T20 resulted in Formulation 6 (with a water content of 25%) – this formulation achieved substantially the same plasma levels as Fasinex® 120 drench (triclabendazole C_{max} of 15.9µg/mL versus 16.5µg/mL) applied at the equivalent dose rate of 12mg/kg. The C_{max} achieved for ivermectin was 2.8ng/mL at 5 days.

Formulation 7 again showed increased bioavailability of triclabendazole when Teric® BL8 was replaced with Ecoteric® T80. The C_{max} achieved for triclabendazole was 12.9µg/mL and the C_{max} achieved for ivermectin was 3.0ng/mL at 2 days.

Having reference to Table 2, in a further feasibility trial (Hereford female weaner cattle, average weight of approximately 200kg, 3 animals per group) reduction of the water content of the formulations to 150g/L, and reverting to Ecoteric® T20 in place of Ecoteric® T80, increased the efficiency of delivery of ivermectin, the ivermectin plasma C_{max} values for the formulations ranging from 8ng/mL to 13ng/mL.

Table 2

Formulation Components	g or mL per litre	Dose Rate mg/kg	AUC	Mean plasma	Plasma C_{max}	T_{max} days
Triclabendazole	90g	9.0	72µg.d/mL	3.6µg/mL	9µg/mL	5
Ivermectin	10.0g	1.0	88ng.d/mL	4.4ng/mL	8ng/mL	5
Ecoteric® T20	200g					
Water	150g					
Benzyl alcohol	30g					
Triethanolamine	5.0g					
Glysolv PM®	608mL					
Triclabendazole	120g	12	85µg.d/mL	4.1µg/mL	12µg/mL	5
Ivermectin	5.0g	0.5	52ng.d/mL	2.5ng/mL	8ng/mL	5
Ecoteric® T20	200g					
Water	150g					
Benzyl alcohol	30g					
Triethanolamine	5.0g					
Glysolv PM®	588mL					
Triclabendazole	180g	18	139µg.d/mL	6.8µg/mL	18µg/mL	5
Ivermectin	7.5g	0.75	79ng.d/mL	4.1ng/mL	13ng/mL	5
Ecoteric® T20	200g					
Water	150g					
Benzyl alcohol	30g					
Triethanolamine	5.0g					
Glysolv PM®	553mL					

From the results provided in Tables 1 and 2, it is apparent that the pharmacokinetics of the active agents can be altered as desired by manipulating the water content, and the type and content of the surfactant and/or the co-solvent used in micellar formulations according to the invention.

Manipulation of the solvent and co-solvent type has also been found during the course of these experiments to affect the physical stability of the micellar formulations, use of a combination of Butyl diGlysolv® and PEG 200 providing the best cold storage stability and highest maximum concentration for triclabendazole of the formulations tested, thereby providing a more rugged product suitable for application to animals in the cooler months of late autumn or early spring - although there is no published data, it has been reported that greater amounts of active components need to be applied to animals in colder months to get the required efficacy, and these months are typically the most important in liver fluke control.

Example 3 – Dosing studies

Example 3.1 – Concentration effect (constant volume)

Having reference to Table 2, it can be seen that altering the concentration of triclabendazole and/or ivermectin in the aqueous micellar formulations of the invention provides a corresponding change in AUC, when applied to the animal in the same volume of formulation (1mL applied/ 10kg animal).

Example 3.2 – Concentration effect (constant dose)

Having reference to Table 3, in a critical slaughter efficacy trial of formulations according to the invention (methods as per Example 2; mixed sex Hereford weaner cattle, average weight of approximately 200kg, 5 animals per group), an aqueous micellar formulation according to the invention comprising triclabendazole at 240g/L, but varying ivermectin concentration was applied at a constant ivermectin dosage rate (0.5mg/kg), but varying triclabendazole dosage rate (12 to 36mg/kg).

The results show that application of a more concentrated ivermectin dose in a smaller volume (same final ivermectin dose rate), resulted in improved pharmacokinetic results, including greater C_{max} and/or greater bioavailability (AUC) of the ivermectin.

Table 3

Formulation Components	g or mL per litre	Dose Rate mg/kg	Dose Rate mL/kg	AUC	Plasma C_{max}	T_{max} days
Triclabendazole	240g	12	1ml/20	73µg.d/mL	8.3µg/mL	5
Ivermectin	10g	0.5	1ml/20	104ng.d/mL	10.4ng/mL	7
Ecoteric® T20	200g					
PEG 200	30g					
Water	150g					
Triethanolamine	0.74g					
Brilliant Blue FCF	0.16g					
Butyl di Glysolv®	491mL					
Triclabendazole	240	24	1ml/10	129µg.d/mL	15.1µg/mL	5
Ivermectin	5	0.5	1ml/10	84ng.d/mL	9.5ng/mL	5
Ecoteric® T20	200					
PEG 200	30					
Water	150					
Sodium dodecyl sulphate	20					
Brilliant Blue FCF	0.16					
Butyl di Glysolv®	480 mL					
Triclabendazole	240g	36	1ml/6.67	177µg.d/mL	18.6µg/mL	7
Ivermectin	3.33g	0.5	1ml/6.67	82ng.d/mL	7.5ng/mL	7
Ecoteric® T20	200g					
PEG 200	30g					
Water	150g					
Triethanolamine	1.12g					
Brilliant Blue FCF	0.16g					
Butyl di Glysolv®	498mL					

In another trial (also carried out as described in Example 2), a formulation according to the invention having 180g/L triclabendazole and 7.5g/L ivermectin, and a formulation having 240g/L triclabendazole and 10g/L ivermectin, were applied to animals over different area sizes on the backs of the animals (from the middle of the back towards the rump), while maintaining the same dose rate for the active constituents. The results, shown in Table 4, show that application of the ivermectin and triclabendazole in a higher concentration formulation applied over a smaller area makes the active agents more bioavailable.

TABLE 4

Formulation details	g or mL per litre	Dose rate (mg/kg)	Mean Treatment Area (cm^2)	Mean plasma conc^n	AUC
Triclabendazole	180	12.0	110	3.3µg/mL	65µg.d/mL
Ivermectin	7.5	0.5		1.7ng/mL	30ng.d/mL
Ecoteric T 20®	200	(1mL/ 15 kg)			
PEG 200	30				
Water	150				
Triethanolamine	0.15				
Brilliant Blue FCF	0.16				
Butyl diGlysolv®	536mL				
Triclabendazole	240	12.0	76	5.1µg/mL	170µg.d/mL
Ivermectin	10.0	0.5 (1mL/20kg)		2.2ng/mL	43ng.d/mL
Ecoteric T 20®	200				
PEG 200	30				
Water	150				
Triethanolamine	0.3				
Brilliant Blue FCF	0.16				
Butyl diGlysolv®	500mL				

Example 4 – Stability studies

Samples of formulation A, the composition and preparation of which is described in Example 1, which contains sodium dodecyl sulphate, were stored at 4, 30 and 40°C in 250 mL high density polyethylene bottles sealed with screw caps, sampled at 1, 2 and 3 months, and tested for ivermectin and triclabendazole content. Triclabendazole and ivermectin content of the formulations was determined using validated stability indicating methods based on reversed phase HPLC with UV detection. The results, provided in Table 5, demonstrate the chemical stability of the formulation at accelerated storage conditions – effectively no degradation of the active components occurred even after 6 months storage at 40°C.

TABLE 5

Storage Temperature	Triclabendazole Content (g/L) after storage time:				Ivermectin Content (g/L) after storage time:			
	1 month	2 months	3 months	6 months	1 month	2 months	3 months	6 months
4°C	250	248	247	241	7.55	7.53	7.84	7.53
30°C	247	248	247	240	7.47	7.52	7.77	7.49
40°C	247	249	241	242	7.45	7.55	7.71	7.41

In another stability trial a number of substances were tested for their potential as a stabiliser for the formulations, ivermectin being unstable in inadequately stabilised formulations. The substances were each tested at a concentration of 10.0 g/L, except phosphate buffers, in a formulation otherwise having the following composition (per Litre):

Triclabendazole	120g
Ivermectin	5.0g
Teric BL 8®	200g
Benzyl alcohol	30g
Water	150g
Brilliant Blue FCF	0.16g
Butyl Di Glysolv®	485 mL

The samples were stored at 50°C in 250 mL high density polyethylene bottles sealed with screw caps, and sampled at 3 months, and tested for ivermectin and triclabendazole content. Triclabendazole and ivermectin content of the formulations was determined using validated stability indicating methods based on reversed phase HPLC with UV detection. The data, provided in Table 6, illustrate the difficulty of stabilising the ivermectin component of the formulation.

From the stability data it was concluded that inclusion of anionic surfactants such as the linear alkyl sulphate sodium dodecyl sulphate, or buffering agents such as one or more monobasic/ dibasic phosphates, or mixtures thereof, in the formulations of the invention significantly improve the stability of the ivermectin component.

Table 6

Candidate Stabiliser	g/L	Triclabendazole Content (g/L) after storage time:		Ivermectin Content (g/L) after storage time:		
		Initial	3 months @ 50°C	Initial	3 months @ 50°C	% Ivermectin Breakdown
-	-	124.1	122.0	4.96	4.33	12.7
Butylated hydroxy toluene (BHT)	10.0	123.8	122.5	4.92	4.36	11.4
Epoxidised Resin (ERL 4221)	10.0	123.2	123.2	4.89	3.45	29.4
Vitamin E Acetate	10.0	123.1	122.2	4.87	4.36	10.5
Triethanolamine	10.0	121.7	122.4	4.70	1.88	60.0
Disodium hydrogen phosphate	0.18	110.0	109.5	4.39	4.24	3.4
Dihydrogen sodium phosphate	1.57					

Example 5 – Efficacy studies

Materials and Methods

Cattle (typically Hereford or Hereford cross breed) with either natural or artificially infected burdens of fluke and nematodes were used in pen and field trials. They were allotted into treatment groups, each having similar mean weights and fluke and nematode burdens. Experimental treatments were applied along the backline from the middle of the back towards the rump, using a commercially available backliner gun fitted with a plastic shroud to ensure correct delivery of the formulation according to the protocol.

Efficacy was measured by either decrease in faecal egg counts over time or total parasite counts from gastrointestinal tracts and livers recovered after slaughter. The reported data are based on group arithmetic and/or group geometric means.

Efficacy based on faecal worm egg counts were calculated as follows:

$$\% \text{ Efficacy} = 100 [1 - (T_2 C_1 / T_1 C_2)]$$

where T, C, 1 and 2 refer to treated, control, pre-treatment and post treatment mean worm egg counts respectively.

All other Efficacy data were calculated using the formula:

%Efficacy = 100(C-T/C)

where T and C refer to treated and control mean total worm counts respectively.

For critical slaughter nematode efficacy studies, the animals were slaughtered at 14 or 21 days post treatment.

For critical slaughter efficacy studies against all stages of the liver fluke (artificially infested), the animals were slaughtered 100 days after treatment.

Results

Example 5.1

A critical slaughter pen efficacy trial (naturally acquired fluke and nematodes) involved mixed sex Hereford and Hereford/Angus cross weaned calves selected from 2 large commercial herds. The animals were randomly allocated to groups of 5 animals such that each group had a similar mean and range of *Fasciola hepatica* egg counts and body weights. Prior to treatment, animals were moved to a research feedlot to avoid further infection. At treatment the animals were weighed and treated with formulations of the triclabendazole + ivermectin pour on administered at different dose volumes and active concentrations. One group of 5 animals remained as untreated negative control.

All animals were slaughtered 19 to 21 days post treatment, gastrointestinal tracts and livers recovered, and total worm and fluke numbers determined.

Treatment formulations involving different concentrations of active components and/or different excipients were tested, these formulations being as follows:

Group 1	g or mL/L	Dosage rate (mg/kg)
Triclabendazole	240g	12
Ivermectin	10.0g	0.5
Ecoteric T20®	200g	
PEG 200	30g	
Water	150g	
Triethanolamine	0.74g	
Brilliant Blue FCF	0.16g	
Butyl diGlysolv®	491mL	

	g or mL/L	Dosage rate (mg/kg)
Group 2		
Triclabendazole	240g	24
Ivermectin	5.0g	0.5
Ecoteric T20®	200g	
PEG 200	30g	
Water	150g	
Triethanolamine	1.27g	
Brilliant Blue FCF	0.16g	
Butyl diGlysolv®	494mL	
Group 3		
Triclabendazole	240g	36
Ivermectin	3.33g	0.5
Ecoteric T20®	200g	
PEG 200	30g	
Water	150g	
Triethanolamine	1.12g	
Brilliant Blue FCF	0.16g	
Butyl diGlysolv®	498mL	
Group 4		
Triclabendazole	240g	24
Ivermectin	5.0g	0.5
Ecoteric T20®	180g	
PEG 200	30g	
Water	150g	
Brilliant Blue FCF	0.16g	
Sodium dodecyl sulphate	20g	
Butyl diGlysolv®	480mL	
Group 5		
Triclabendazole	240g	24
Ivermectin	5.0g	0.5
Ecoteric T20®	200g	
PEG 200	30g	
Water	150g	
Brilliant Blue FCF	0.16g	
Sodium dodecyl sulphate	20g	
Butyl diGlysolv®	480mL	
Group 6		
Triclabendazole	240g	24
Ivermectin	5.0g	0.5
Ecoteric T20®	200g	
PEG 200	30g	
Water	150g	
Brilliant Blue FCF	0.16g	
Sodium dodecyl sulphate	20g	
Butyl diGlysolv®	316mL	
Ethylene glycoldiacetate	155mL	

The results, provided in Table 7, show that effective control of flukes and nematodes is achievable using a practical volume of an aqueous micellar pour-on formulation of the present invention.

TABLE 7

% Treatment efficacy against parasites
(values based on the geometric mean of total worm count are given in brackets where different to those based on the arithmetic mean)

Group No.	Liver	Abomasum		
	F. hepatica (adult)	*H.contortus* (adult)	*Ostertagia spp* (adult)	*T. axei* (adult)
1	100	>99.9	>99.9	>99.9
2	100	>99.9	98.2 (96.4)	>99.9
3	100	>99.9	95.8 (86.6)	>99.9
4	100	>99.9	89.1 (81.8)	>99.9
5	100	>99.9	>99.9	>99.9
6	100	>99.9	69.2 (91.9)	>99.9

Group No.	Small intestine				
	Trichostrongylus spp (adult)	*Cooperia spp* (adult)	*Cooperia spp* (immature)	*Cooperia spp* L4	*Nematodirus spp* (adult)
1	94.4	88.5 (96.7)	>99.9	92.3 (85.9)	negative
2	54.9 (negative)	56.1 (66.4)	>99.9	>99.9	negative
3	85.9 (84.9)	91.4 (88.3)	>99.9	>99.9	50 (18.5)
4	57.7 (93.8)	80.2 (84.3)	>99.9	>99.9	25 (8)
5	92.5 (96.1)	89.8 (98.7)	>99.9	>99.9	>99.9
6	91.5 (88.3)	36.3 (83.6)	>99.9	53.8 (75.8)	>99.9

Group No.	Large intestine	
	Oesophagostomum (adult)	*Trichuris* (adult)
1	>99.9	99.9 (>99.9)
2	>99.9	14.3 (negative)
3	>99.9	99.9 (>99.9)
4	>99.9	99.9 (>99.9)
5	>99.9	85.7 (71.2)
6	>99.9	85.7 (71.2)

The product was 100 % effective against adult *Fasciola hepatica* at dose rates of 12, 24 and 36 mg/kg triclabendazole and effective against nematodes at a dose rate of 0.5 mg/kg ivermectin. In this trial, an effective treatment of animals for

endoparasites was achieved using 1mL/ 20kg of a formulation including 240g/L triclabendazole and 10.0g/L ivermectin (12mg/kg triclabendazole and 0.5 mg/kg ivermectin).

Example 5.2

Two critical slaughter studies were designed to compare the efficacy of a formulation according to the invention (see below) against immature and adult stages of the liver fluke *Fasciola hepatica*, and naturally acquired roundworm infections in cattle. The efficacy of the triclabendazole + ivermectin pour-on against immature and mature stages of *Fasciola hepatica* based on arithmetic mean was 70.5% and 99.2% respectively. Control of gastrointestinal strongyles by the test formulation (Group 5, Example 5.1, Table 8) as assessed using total worm counts at slaughter was 86% to 99.9% (arithmetic mean) for nematodes found in the abomasum, small and large intestines.

Test formulation - described in Example 1.1, Formulation A

Component	**g or mL/L**	**Dose Rate (mg/kg)**
Triclabendazole	240g	24.0
Ivermectin	7.5 g	0.75
Ecoteric T20®	200g	
PEG 200	30g	
Water	150g	
Brilliant Blue FCF	0.16g	
Sodium dodecyl sulphate	20g	
Butyl diGlysolv®	475 mL	

Example 5.3

Three field trials (faecal egg count reduction tests) were designed to determine the efficacy of the formulation described in Example 5.2 under field conditions. Sixty cattle were split into groups of 15, one of the groups remaining as an untreated control. Good efficacy of the formulation against *Fasciola hepatica* as assessed by a reduction in faecal egg counts as compared to the untreated controls of >90% (AM) was reported in all trials 14 days post treatment.

Industrial Applicability

The formulations of the invention can be readily used to treat, control or prevent disease caused by, and/or infestations of, endo-parasites such as liver fluke and nematodes as well as ecto-parasites, particularly in treating, controlling and/or preventing liver fluke and nematode infestations in sheep or cattle, particularly cattle.

It will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention as defined in the following claims.

The claims defining the invention are as follows:

1. An aqueous micellar formulation comprising a first active agent selected from water insoluble benzimidazoles, salicylanilides and active derivatives or salts thereof in combination with a second active agent selected from macrocyclic lactones or active derivatives or salts thereof, said formulation being for topical application to animals for the control of internal parasites and also comprising, per litre of formulation:

from about 100 to about 400g veterinary acceptable surfactant(s);

from about 200 to about 750g veterinary acceptable water-miscible solvent(s); and

from about 50 to about 350g water.

2. A stable formulation according to claim 1, wherein said surfactant is selected from sorbitan esters, polyoxyalkylated sorbitan esters, polyoxyalkylated alkyl ethers, polyoxyalkylated fatty alcohols, polyalkylene glycol esters, polyoxyalkylated derivatives of castor oil, polyglycerol esters, polyoxyalkylated fatty alcohols, polyoxyalkylated fatty acids, copolymers of ethylene oxide and propylene oxide; amine ethoxylates; alkyl phenol ethoxylates; alkyl polysaccharides; linear alkylbenzene sulphonates; C12 to C16 alcohol sulphates; C12 alkoxypolyethanoxy sulphates; alkyl phosphates and phosphonates, or combinations thereof.

3. A formulation according to claim 2, wherein said surfactant is selected from polyoxyethylene sorbitan fatty acid esters or combinations thereof.

4. A formulation according to claim 3, wherein said surfactant is polyoxyethylene (20) sorbitan monolaurate.

5. A formulation according to any one of claims 1 to 4, wherein said water miscible solvent is selected from ethanol, isopropanol, benzyl alcohol, glycol ethers, liquid polyoxyethylene glycols or a mixture of at least two of these solvents.

6. A formulation according to claim 5, wherein the glycol ethers is/are selected from alkylene or dialkylene glycol monoalkyl ethers.

7. A formulation according to claim 6, wherein said glycol ether(s) is/are selected from propylene glycol monomethyl ether, diethylene glycol monoethyl ether, and diethylene glycol monobutyl ethers.

8. A formulation according to claim 6 or claim 7 comprising a glycol ether in combination with benzyl alcohol or a liquid polyethylene glycol as water-miscible solvent.

9. A formulation according to claim 8 comprising a glycol ether and a liquid polyethylene glycol as water-miscible solvents.

10. A formulation according to claim 9, wherein the polyethylene glycol is PEG 200.

11. A formulation according to any one of claims 1 to 10 further comprising a stabiliser selected from anionic surfactants, buffering agents and mixtures thereof.

12. A formulation according to claim 11 wherein said stabiliser is selected from linear alkyl sulphates, linear alkyl benzene sulphonates, and phosphates.

13. A formulation according to claim 11 wherein said stabiliser is selected from sodium dodecyl sulphate, a mixture of mono- and di-basic phosphates, or mixtures thereof.

14. A formulation according to claim 11 wherein said stabiliser is sodium dodecyl sulphate.

15. A formulation according to any one of claims 1 to 14, comprising about 150 to about 250g surfactant per litre of formulation.

16. A formulation according to any one of claims 1 to 15, comprising from about 300 to about 650g water-miscible solvent(s) per litre of formulation.

17. A formulation according to claim 16, comprising about 500 to about 550g water-miscible solvent per litre of formulation.

18. A formulation according to any one of claims 1 to 15, comprising from about 300 to about 650g glycol ether(s) selected from alkylene or dialkylene glycol monoalkyl ethers and from about 10 to about 100g of benzyl alcohol or a liquid polyethylene glycol, or a combination thereof as water-miscible solvent(s) per litre of formulation.

19. A formulation according to claim 18, comprising about 450 to about 550g glycol ether(s) selected from alkylene or dialkylene glycol monoalkyl ethers and about 20 to about 50g of benzyl alcohol or a liquid polyethylene glycol, or a combination thereof as water-miscible solvent(s) per litre of formulation.

20. A formulation according to any one of claims 1 to 19, comprising about 150g water per litre of formulation.

21. A formulation according to any one of claims 1 to 20, wherein said first active agent comprises one or more benzimidazoles selected from thiabendazole; albendazole; albendazole sulfoxide or albendazole oxide; cambendazole; fenbendazole; mebendazole; oxfendazole; oxibendazole; parbendazole; cyclobendazole; flubendazole; triclabendazole; and luxabendazole, or active derivatives or salts thereof.

22. A formulation according to claim 21, wherein said first active agent is triclabendazole.

23. A formulation according to any one of claims 1 to 22, comprising from about 120 to about 300g benzimidazole, or a derivative thereof, per litre formulation.

24. A formulation according to claim 23, comprising from about 180 to about 240g benzimidazole, or derivative thereof, per litre formulation.

25. A formulation according to any one of claims 1 to 24, wherein said macrocyclic lactone is selected from abamectin, ivermectin, eprinomectin, doramectin, moxidectin, selamectin or milbemycins, or active derivatives or salts thereof.

26. A formulation according to claim 25, wherein said macrocyclic lactone is ivermectin.

27. A formulation according to any one of claims 1 to 26, comprising from about 5 to about 10g macrocyclic lactone per litre formulation.

28. A formulation according to claim 27, comprising about 7.5g macrocyclic lactone per litre formulation.

29. An aqueous micellar formulation comprising a benzimidazole in combination with a macrocyclic lactone, said formulation being for topical application to animals for the control of internal parasites and also comprising, per litre of formulation:

about 100 to about 300g polyoxyalkylene sorbitan fatty acid ester surfactant;

about 300 to about 650g alkylene glycol ether selected from alkylene or dialkylene glycol monoalkyl ethers or combinations thereof;

about 10 to about 100g liquid polyethylene glycol;

about 5 to about 50g stabiliser selected from linear alkyl sulphates, linear alkyl benzene sulphonates, and phosphates; and

about 50 to about 350g water.

30. A formulation according to claim 29 wherein said stabiliser is selected from sodium dodecyl sulphate, a mixture of mono- and di-basic phosphates, or mixtures thereof.

31. A formulation according to claim 29 wherein said stabiliser is sodium dodecyl sulphate.

32. A formulation according to claim 29 comprising, per litre formulation:

about 180 to about 240g triclabendazole;

about 5 to about 10g macrocyclic lactone or an active derivative or salt thereof;

about 150 to about 250g polyoxyethylene (20) sorbitan monolaurate;

about 450 to about 550g diethylene glycol monobutyl ether;

about 20 to about 50g PEG 200;

about 20g sodium dodecyl sulphate; and

about 100 to about 200g water.

33. A method of treating or preventing a diseased or parasite infested state in a mammal, comprising topically administering to said mammal a micellar formulation according to any one of claims 1 to 32.

34. A method according to claim 33, for treatment or prevention of at least a liver fluke infection/infestation in a mammal.

35. A method according to claim 33, for treatment or prevention of at least a nematode infection/infestation in a mammal.

36. A method according to claim 33, for treatment or prevention of at least a liver fluke and a nematode infection/infestation in a mammal.

37. A method according to any one of claims 33 to 36, wherein said mammal is selected from livestock.

38. A method according to claim 37, wherein the livestock is selected from cattle, sheep, goats, pigs and horses.

39. A method according to any one of claims 33 to 38, wherein said formulation is a formulation according to any one of claims 29 to 32.

40. A method according to any one of claims 33 to 39, wherein said topical application comprises application of the formulation in a band along the lower portion of the back of the mammal.

41. A method according to claim 40, wherein the formulation is applied to the mammal over as small a region as possible while avoiding run-off of the

formulation so as to maximise the concentration of active agents per cm^2 of animal surface.

42. A method according to claim 40 or claim 41, wherein the formulation is sprayed onto the back of the mammal.

43. A method according to any one of claims 40 to 42 wherein the mammal is selected from cattle, and the band of formulation is applied starting from the thoracic vertebrae and proceeding towards the rump of the animal, and about 24mg benzimidazole and about 0.75mg macrocyclic lactone are applied per kilogram animal.

44. A method according to any one of claims 40 to 43, wherein said band is about 5 to about 15 cm wide and about 20 to about 40 cm long.

45. A method according to any one of claims 40 to 44, wherein the formulation is sprayed onto the back of the animal and the height of the source of spray relative to the back of the animal is maintained at about 15cm.

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